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Effects of development, temperature, and calcium hypochlorite treatment on *in vitro* germinability of *Phalaenopsis* seeds

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ABSTRACT

There are no standardized procedures for sanitizing orchid seeds for propagation by tissue culture and there is insufficient information about the optimum stage of orchid seed development for best germination. *Phalaenopsis amabilis* flowers were hand-pollinated and fruits harvested 90, 105, and 120 d after pollination (DAP) for seed developmental analysis. Embryo cell number per seed was counted after staining with 4′-6-diamidino-2-phenylindole and viewing through a confocal microscope. Germination percentage and cell number per embryo increased from 14 to 61% and 41 to 66%, respectively, during fruit development from 90 to 120 DAP. Seeds from mature, browning (~140 DAP) *Phalaenopsis* Sogo Lit-Angel and *Phalaenopsis* spp. breeding line 9450 seed pods failed to germinate until frozen at −196, −80, or −18 °C and thawed or chilled at 4 °C for 10 d. Germinability in 140 DAP seeds was correlated with cracked testa after freezing and thawing. *P. amabilis* seeds were treated with 0, 5, 10, or 15% calcium hypochlorite (CH) for 5, 10, or 15 min. Ninety six percent of untreated seeds from 90 DAP fruit produced protocorms within 40 d after sowing (DAS). Exposing seeds to 5% CH for 10 or 15 min decreased germination to 85 and 73%, respectively. Exposure to 10 or 15% CH for 5, 10, or 15 min produced seed germination percentages of less than 40%. Protocorms developed root hairs and shoot primordia by 50 DAS and an average of one leaf and root by 85 DAS after treatment with either 0 or 5% CH. Higher concentrations delayed or inhibited protocorm development. Green fruits 120 DAP produced the highest percentage of protocorms, while ~140 DAP seeds from browning fruit were dormant but cold treatments increased germination.

1. Introduction

The development of orchid ovules follows a pattern different from most angiosperms. At pollination, there are no developed ovules in most orchid species. According to Nadeau et al. (1996), hormonal signaling associated with pollination leads to ovule development in *Phalaenopsis*. *Phalaenopsis* species commit to ovule development between 28 and 48 d after pollination (DAP), and ovule development is nearly complete 77 d after pollination when fertilization generally occurs (Nadeau et al., 1996). Other studies have verified that fertilization occurs 70–120 d after pollination and embryo development begins within about 4 weeks (Arditti, 1992; Rasmussen, 1995). Because of the small size of *Phalaenopsis* orchid seeds, about 0.4 mm × 0.08 mm (McKendrick et al., 2000), and the long developmental time after pollination (Popova et al., 2003), few developmental studies have been conducted.

Immature orchid embryos germinate more readily *in vitro*. Immature *Cypripedium* seeds, containing embryos approximately 66% of mature size with only 9–12 cells and a living testa, germinated better than fully mature seeds (Rasmussen, 1995). Immature seeds may germinate well because of efficient protein mobilization during rehydration and an undeveloped embryonic envelope (Rasmussen, 1995). However, mature seeds may have greater potential for propagation and storage (Miyoshi and Mii, 1998). Because there is no consensus on the optimum stage of development for best seed quality in *Phalaenopsis amabilis*, a seed developmental study was conducted. Nuclear staining marked embryo cells that were counted using confocal fluorescence microscopy throughout development, since traditional techniques like fresh and dry weight determination were impractical for orchid. We focused on developing pods between 90 and 120 DAP when seed cell number was rapidly increasing (Vujanovic et al., 2000).

Of the world's 25,000 orchid species, some 10% are endangered due to high collection pressure and loss of habitat (Sharma et al., 2003). There are currently very few genebanks that preserve orchid

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germplasm as seeds and procedures to improve orchid seed storage for germplasm conservation are needed. An additional objective of this study was to test the tolerance of orchid seed to rapid freezing in liquid nitrogen to determine whether cryopreservation may be a viable long-term strategy for orchid germplasm preservation.

Orchids are mass-produced commercially by germinating sanitized seeds in aseptic culture. Sodium or calcium hypochlorite (CH) solutions are often used to reduce contamination in culture and stimulate seed germination. CH is very effective at lower concentrations and short exposures (Blischak, 2005). Stimulatory effects of CH have been reported in some orchid species as well. Ervin and Wetzel (2002) observed a 50% reduction in mean time to germination of *Juncus effusus*, while Vujanovic et al. (2000) also observed increased germinability of *Cypripedium* spp. seeds following treatment with CH. Miyoshi and Mii (1998) reported that germination of *Cypripedium macranthos* was stimulated after treatment with sodium hypochlorite (1% available chlorine) for 15–30 min and CH (3.2% available chlorine) for 5–7 h. These studies suggest seed genotype, concentration of sanitizing agent, and duration of exposure are important factors affecting both seed germinability and the level of contamination in orchid seed cultures. This study was conducted in collaboration with the Ornamental Plant Germplasm Center of the United States Department of Agriculture who has a mandate to collect and preserve orchid germplasm using seeds. Although commercial orchid producers may have proprietary sterilization and propagation techniques, there is a need for more public information particularly pertaining to harvesting, handling, storing, and propagating for germplasm preservation, especially when seeds are collected from dehisced pods that are badly contaminated. The ability of various treatment durations and concentrations of CH to control microbial contamination and affect germination and seedling development of cultured *P. amabilis* seeds was evaluated.

2. Materials and methods

2.1. Plant material

This study was conducted using seeds from multiple genotypes produced both in Taiwan and in Virginia. Twenty-five uniform budding mericlonal *P. amabilis* plants were purchased (The Orchid Station, Barboursville, VA) and grown for seed production in a shaded (40% light transmission) greenhouse with maximum and minimum temperatures of 30 and 19 °C, respectively, in Blacksburg, VA from December through May. Flowers were hand-pollinated by removing the anther cap and pollinia with forceps and then placing the pollinia on the stigma. Dr. Fu-chi Chen of National Pingtung University, Taiwan provided seeds of *Phalaenopsis* Sogo Lit-Angel and *Phalaenopsis* spp. breeding line 9450.

2.2. Harvesting

Seed capsules were harvested at 90, 105, and 120 DAP when seed development and cell number increased rapidly (Vujanovic et al., 2000). Stems were uniformly cut with a knife approximately 1 cm below the pod attachment. Pods were placed in self-sealing plastic bags for transport and short-term storage to minimize desiccation.

2.3. Seed germination and viability testing

Mature seeds from Taiwan were harvested from browning seed pods approximately 140 DAP, stored at 4 °C for 3 months and hand-carried to the US. Upon arrival, seeds were placed in microcentrifuge tubes, mixed with 1 mL of 15% (w/v) CH (3.7% chlorine), vortexed for 7 min, and incubated for an additional 8 min. Seeds were spread on Petri dishes containing Phytamax Orchid Maintenance Medium (Sigma–Aldrich) supplemented with filter-sterilized coconut water. Petri dishes were incubated at 20 °C for 40 d and seeds that developed into protocorms were counted as germinated. Germination percentages for each treatment were averaged from three grids (1 cm^2) randomly selected on each Petri plate. Grids typically contained between 20 and 50 seeds each.

Seed viability was assessed using tetrazolium triphenyl chloride (TZ) staining. Seeds were pre-soaked on moist filter in 60 mm Petri dishes for 6 h at 25 °C then 15 mL of 1% (w/v) 2,3,5-triphenyl-2H-tetrazolium chloride, adjusted to pH 6.5 with NaOH, was added and the mixture incubated in the dark at 30 °C for 24 h (ISTA, 1985). Seeds were viewed under a microscope and pink and red stained seeds were considered viable. Viability percentages from three grids randomly selected on each Petri plate were calculated and averaged as described above for germination testing.

2.4. Seed staining and confocal microscopy

Groups of seeds from at least three pods were stained for 10 (90 DAP), 90 (105 DAP), or 180 min (120 DAP) at 4 °C in an aqueous 0.1 mg mL^{-1} solution of 4′-6-diamidino-2-pheylindole (DAPI), a stain specific for nuclei, and at least 20 seeds from each pod were visualized using a confocal microscope (Zeiss LSM 510 Laser). Fluorescing nuclei were counted at each stage of development to determine embryo cell number. For lipid detection, whole orchid seeds were stained with Sudan Black, sectioned by hand with a razor blade, and viewed under a light microscope (Bayliss and Adams, 1972).

2.5. Temperature treatments

The moisture content of *P.* Sogo Lit-Angel and *Phalaenopsis* spp. line 9450 seeds was adjusted to 45.5% RH over saturated chromium dichromate solution and submerged in liquid nitrogen (−196 °C) for 30 min and thawed in a water bath (20 °C) for 1 min prior to germination testing. Other seeds were exposed to −80, −18, 4, or 25 °C for 10 d prior to germination testing for 40 d as described above. Two replications of at least 25 seeds each were stained with 0.1% (w/v) Toluidine O Blue (Merck) in distilled water and viewed under a light microscope. Data on protocorm, leaf, and root development were recorded 50 and 85 d after sowing (DAS).

2.6. Capsule and seed sterilization

Ninety DAP pods were surfaced treated with 15% CH for 30 min then opened with an alcohol-flamed scalpel in a laminar-flow hood. Seeds were gently teased from the pod with the scalpel and plated directly on Phytamax Media or treated with 5, 10, or 15% CH for 5, 10, or 15 min before plating. Untreated seeds were harvested from surface sterilized pods and plated. Germination data were collected as described above.

2.7. Statistical analysis

Each treatment was replicated three times in a Completely Randomized Design. Data were analyzed using the SAS statistical software and means separated by $LSD_{0.05}$. Graphs and linear regressions were performed using Microsoft Excel software. Standard error (S.E.) was calculated to show differences among developmental means.

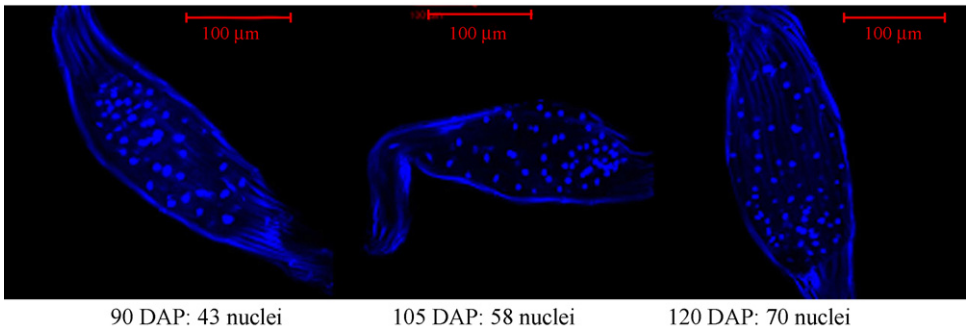

Fig. 1. Visualization of orchid embryo cell number during development. Nuclei stained blue with 0.1 mg mL^{-1} (in water) 4′-6-diamidino-2-phenylindole (DAPI) could be counted using confocal microscopy. Mean embryo cell number increased with pod maturity as summarized in Table 1. Representative are seeds shown for each stage of development. 90 DAP: 43 nuclei 105 DAP: 58 nuclei 120 DAP: 70 nuclei. Scale: 100 μm.

3. Results

Mean pod weight and length of *P. amabilis* did not increase between 90 and 120 d but pod diameter increased between 90 and 105 d, indicating basic seed pod dimensions were established earlier in development (Table 1). Seeds were stained with DAPI to determine the mean number of embryo cells in developing seeds, which increased from 41 to 66 from 90 to 120 DAP, respectively, as pod diameter also increased (Table 1, Fig. 1).

At 120 DAP, DAPI staining was less effective and required addition incubation to obtain the same positive staining of nuclei as 90 DAP. This suggested that permeability of the testa or embryo envelope also decreased during development, slowing the diffusion of DAPI to the embryo. To test this hypothesis, seeds were treated with 80% ethanol for 24 h, prior to staining, which reduced incubation times to about the same as required for 90 DAP seeds. To visualize the putative impermeable carapace layer in the orchid testa, seeds were stained with Sudan Black, a lipid specific stain. Positive staining in seeds suggested lipophilic development in the testa after 90 DAP (data not shown).

P. amabilis seeds germinated 14% at 90 DAP and there was a linear increase in germination percentage to 61% at 120 DAP (Fig. 2). At 90 DAP, 41% of seeds gave a positive TZ test, and this increased to 65% at 120 DAP as seeds matured (Figs. 2 and 3). Two additional genotypes of fully mature (~140 DAP) *Phalaenopsis* orchid seed lots were tested for both viability and germinability. Positive TZ staining occurred in 51 and 84% of seeds of *P.* Sogo Lit-Angel and line 9450, respectively (Table 2), and distinctive pink or red staining patterns were observed (Fig. 3). Germination of *P.* Sogo Lit-Angel and line 9450 was 0 and 2.8%, respectively, after 120 d, suggesting that a significant percentage of mature *Phalaenopsis* seeds were dormant (Table 2).

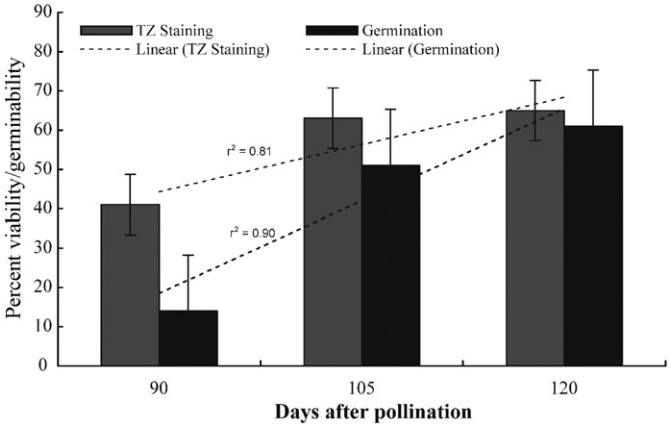

Fig. 2. *P. amabilis* seed germinability *in vitro* and TZ staining during development. TZ staining estimated higher viability than germination tests, which were conduced for 120 d. Both TZ staining and *in vitro* germinability increased with maturity, suggesting that mature seeds lack dormancy.

Table 1
P. amabilis pod weight, length, diameter, color, and nuclei number 90, 105, and 120 DAP

Pod characteristics at harvest					
DAP days	Mean pod weight (g)	Mean pod length (cm)	Mean pod diameter (cm)	Pod color	No. of nuclei stained with DAPI
90	3.2a[a]	3.5a	2.0b	Green	41c[b]
105	3.3a	4.0a	3.9a	Green	58b
120	4.1a	4.5a	4.0a	Green	66a

[a] Means represent the average of at least five pods with separation by $LSD_{0.05}$.
[b] Means of at least 20 seeds each from at least three pods.

Table 2
Viability, determined by red coloration after treatment with 1% (w/v) 2,3,5-triphenyl-2H-tetrazolium chloride, and germinability, on Phytamax media, of two *Phalaenopsis* seed lots[a]

Seed type	TZ staining (%)	*In vitro* germination (%)	Cracked seed coats before freezing[b] (%)	Cracked seed coats after freezing (%)
P. Sogo Lit-Angel	51b	0a	0a	39b
Phalaenopsis spp. (9450)	84c	2.8a	6.6a	41b

[a] Means represent the average from seeds in three grids (1 cm^2) randomly selected on each Petri plate with separation by $LSD_{0.05}$. Grids typically contained between 20 and 50 seeds each.
[b] Representative examples in Fig. 4, calculated from two replications of 25 seeds each.

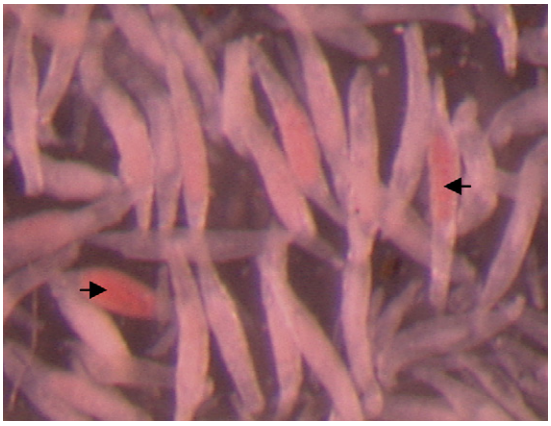

Fig. 3. Seed viability evaluation using TZ staining of mature seeds of *Phalaenopsis* spp. (9450) (100×). Red coloration shows reduction of the dye to an insoluble formazan by hydrogenase complexes in live embryos. Arrows show darker staining in some seeds. TZ percentages for developing seeds are summarized in Fig. 2. Seeds of *P.* Sogo Lit-Angel and *P. amabilis* 120 DAP showed similar pink and red staining.

To further investigate the suitability of *Phalaenopsis* for cryopreservation, short-term freezing or chilling treatments were applied to subsamples of *P.* Sogo Lit-Angel and line 9450. Freezing seeds in liquid nitrogen (−196 °C) and in a freezer at −80 °C increased the germination percentage of *P.* Sogo Lit-Angel as did short-term treatment at 4 °C (Table 3). Due to contamination, no germination percentage was available for line 9450 after liquid nitrogen treatment but 17% of seeds germinated after freezing at −80 °C and thawing and 46% after chilling at 4 °C (Table 3). The testa of 39 and 41% of *P.* Sogo Lit-Angel and line 9450 frozen seeds cracked after freezing and thawing while unfrozen seeds showed less cracking (Table 2, Fig. 4).

Ninety six percent of seeds from 90 DAP pods surface treated with 15% CH and plated directly on germination media without CH seed treatment developed into protocorms with no visible contamination (data not shown). Treating seeds with 5% CH had the least effect on germination as 96% of seeds developed into protocorms, the same percentage as untreated seeds (Fig. 5). Exposing seeds to 5% CH for 10 or 15 min decreased germination to 85 and 73%, respectively. Increasing the concentration to 10% CH dramatically decreased germination percentage regardless of concentration. Exposure for 5, 10, or 15 min produced germination percentages of 27, 40, and 14, respectively (Fig. 5). Exposing seeds to 15% CH for 5, 10, or 15 min produced percentages of 22, 33, and 12, respectively, that were not statistically different from 10% CH (Fig. 5).

Table 3
Protocorm development of seeds adjusted at 45.5% RH and stored at −80, −18, 4, or 25 °C for 10 d or submerged in liquid nitrogen for 30 min, thawed, and planted[a]

Seed type	Storage temperature (°C)					
	−196	−80	−18	4	25	Initial[b]
P. Sogo Lit-Angel	37a	17abc	2bc	23ab	2c	0c
Phalaenopsis spp. (9450)	–	17b	9b	46a	0b	0b

Protocorm development at 40 DAS (%).
[a] Means are the average from seeds in three grids (1 cm^2) randomly selected on each Petri plate with separation by $LSD_{0.05}$ for each genotype. Grids typically contained between 20 and 50 seeds each.
[b] Initial germinability was recorded prior to moisture adjustment or temperature treatment. Cultures contaminated, no data available.

Protocorms were observed for 50 and 85 d after sowing to determine whether CH exposure affected longer-term development after protocorm formation. Exposure to even low concentrations (5%) CH retarded seedling development compared

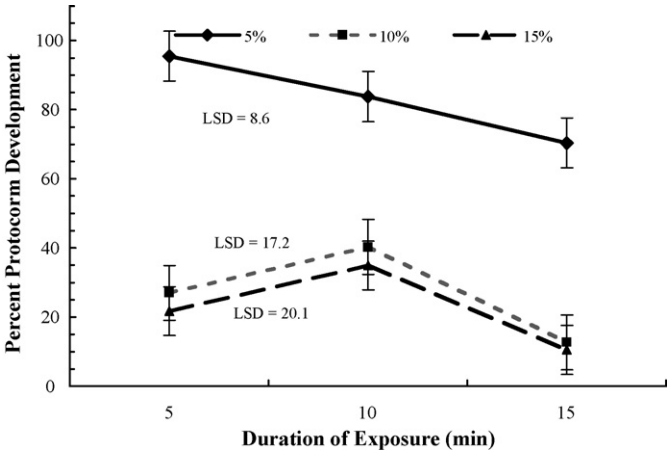

Fig. 5. Protocorm development following treatment of 90 DAP seeds with 0, 5, 10, or 15% CH for 5, 10, or 15 min. Seeds were obtained from 90 DAP pods surface sterilized with 15% CH for 30 min. Protocorm development for untreated seeds receiving only pod decontamination was also 96% (not shown). Error bars are SE averaged from seeds in three grids (1 cm^2) randomly selected on each Petri plate. Grids typically contained between 20 and 50 seeds each. Three plates each were examined for germination until 120 d after sowing.

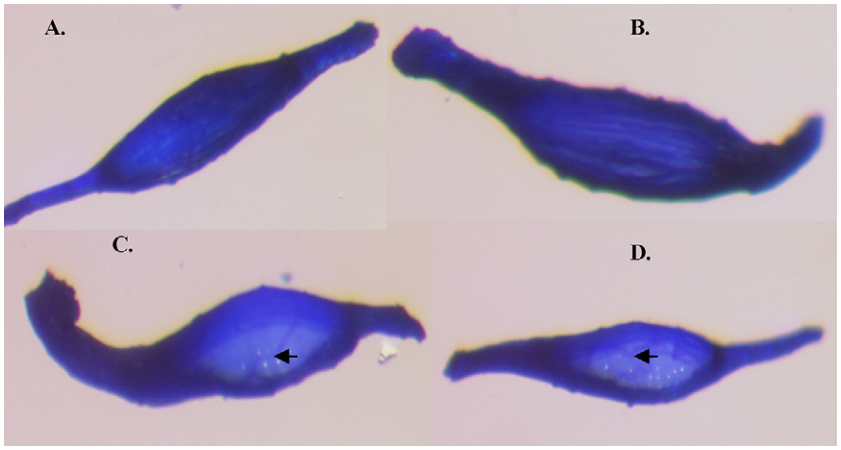

Fig. 4. Effect of liquid nitrogen treatment on the testa of representative *Phalaenopsis* seeds *P.* Sogo Lit-Angel (A and C) or 9450 (B and D). Parts (A and B) are untreated controls; parts (C and D) seeds treated with liquid nitrogen and all seeds were stained with Toluidine O Blue. Arrows show ruptured testa in some seeds after freezing and thawing (240×). Seed cracking data are summarized in Table 2.

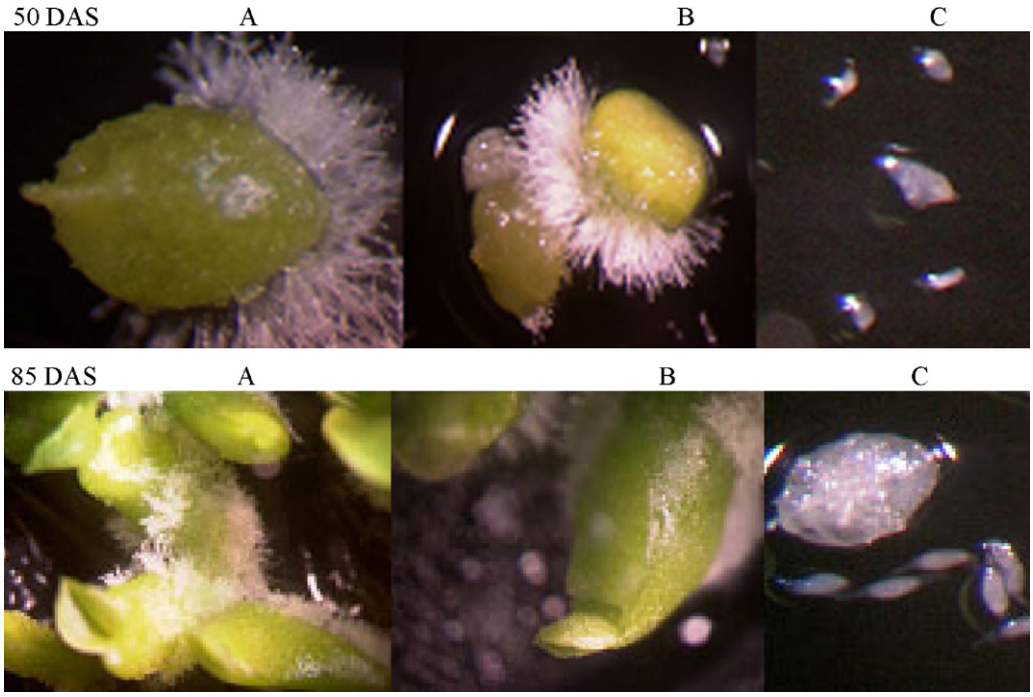

Fig. 6. Representative examples of *Phalaenopsis* seedlings 50 and 85 DAS after seeding 90 DAP seeds. 50 DAS: when only seed pods and not seeds were treated with CH, healthy protocorms with root hairs and shoot primordia were produced (A), seed decontamination with 5% CH for 10 min (B), or seed decontamination with 15% CH for 10 min (C). 85 DAS: an average of one leaf and root developed after treatment with 0 (A) or 5% CH (B). In treatments with 15% CH, only 22% of seeds developed into protocorms without roots or shoots (C).

to untreated seeds (cf. Fig. 6A and B). Seeds exposed to 15% CH showed even greater inhibition of protocorm and subsequent root and shoot development (Fig. 6C).

4. Discussion

Orchid seeds are difficult to study because of their small size and long development time, so conventional characterization of development in terms of fresh and dry mass was not feasible (McKendrick et al., 2000; Popova et al., 2003). Since orchid seeds are very small and translucent, the number of nuclei in individual embryos was counted after DAPI staining using confocal microscopy (Fig. 1). Embryo cell number in *P. amabilis* was a reliable indicator of embryo development and increased to 66 at 120 DAP (Table 1). The cell number in mature orchid embryos reportedly ranges from 8 to 734 (Arditti, 1992).

The combination of hand pollination and greenhouse conditions may have led to a high number of abortions and a plateau in viability of only 65% at 120 DAP. Orchid seed pods developed during winter and early spring under short days and relatively cool greenhouse conditions, which likely slowed development. Pods from all three stages of development were green, while in other studies pod color typically began changing by 120 DAP (Vujanovic et al., 2000). The slow development of orchid exacerbates environmental effects on seed and pod development making it difficult to compare results among studies at different locations based on DAP alone.

In orchid seed development, the inner integument is often been referred to as 'carapace' (Rasmussen, 1995; van der Kinderen, 1995; Yeung et al., 1996). This orchid embryo envelope (testa + carapace) has been described as a multi-cellular layer of dead collapsed cells rich in pectin (Prutsch et al., 2000). Yamazaki and Miyoshi (2006), reported that the carapace in *C. falcate* was comprised of two layers, one lignified and one cutinized and was fully developed by approximately 100 DAP. The carapace may play an important role by isolating seeds from their environment, thus regulating seed germination and physical dormancy. More research is needed to characterize the carapace in orchid seeds.

Studying the carapace was not an original goal of this study, but evidence supports its existence in the three genotypes studied. Although the permeability of DAPI declined during development, <90 DAP seeds imbibed water and swelled the same as less mature seeds (Figs. 1, 3 and 4), consistent with the observation that the embryo envelope in orchid seeds is semipermeable at maturity (Prutsch et al., 2000). Positive staining of free-hand sections of the testa with Sudan Black between 90 and 120 DAP suggests lipophilic development after 90 DAP (not shown). DAPI was less permeable after 90 DAP, possibly due to carapace development, but treatment with nonpolar solvent reduced the incubation time for staining in older seeds (Fig. 1).

Yamazaki and Miyoshi (2006) reported that intact mature *C. falcate* seeds 140 DAP, did not stain with TZ unless the 'carapace' was removed. This indicates that the carapace in mature *C. falcate* seeds was a barrier to TZ diffusion. Darker TZ staining in a small percentage of unfrozen *Phalaenopsis* spp. line 9450 seeds suggests that TZ may have impregnated embryos through minute openings where cutinization and lignification of the carapace was incomplete (Fig. 3). Some seeds appeared darker possibly because the opaque testa was broken and did not obscure the stained embryo (Fig. 3). Only 14% of *P. amabilis* seeds germinated at 90 DAP while 41% stained positively with TZ, suggesting that some seeds exhibited primary dormancy at 90 DAP or were insufficiently developed to germinate (Fig. 2). The highest germination percentage in *C. falcate* seeds occurred 70 DAP and declined with further development (Yamazaki and Miyoshi, 2006). Immature *C. formosanum* seeds germinated better than more mature ones, also suggesting that mature seeds may exhibit primary dormancy (Lee et al., 2005). Nagashima (1994) found that orchid seeds germinated best just as embryogenesis was completed but not later.

When more mature 140 DAP *Phalaenopsis* seeds were examined, none germinated until seed moisture content was adjusted using

saturated salt solutions, and seeds were either frozen and thawing or chilled at 4 °C (Tables 2 and 3). Freezing and thawing cracked the testa of some orchid seeds, which may have increased germination since increased cracking and germination percentage were positively correlated (Tables 2 and 3, Fig. 4). These cracks eliminated the semipermeable barrier between the testa/carapace and the culture media, stimulating germination (Fig. 4). Hirano et al. (2005) suggested that the accumulation of inhibitory substances or increased impermeability of the embryo during maturation might account for low germination in more mature seeds. *C. formosanum* seeds harvested at 135 DAP germinated poorly due to the development of a cuticle in the carapace (Lee et al., 2005). However, chilled seeds in the current study also germinated better without a significant increase in testa cracking, indicating that another mechanism may break dormancy. Incubation at 45.5% RH was common to all treatments but did not increase germination at 25 °C, suggesting that cold treatment was needed. The fact that *Phalaenopsis* seeds survived flash freezing in liquid nitrogen illustrates that cryopreservation of seeds may be a viable strategy for germplasm preservation.

Immature 90 DAP seeds did not germinate well because they were immature or dormant (Fig. 2). Seeds 140 DAP harvested from browning seed pods did not germinate well until chilled, suggesting that at least a fraction of these seeds were dormant as well (Tables 2 and 3). Therefore in this study, 120 DAP seeds harvested from mature green seed pods that had yet to change color produced the best quality seed for propagation and the highest germination percentages without treatment (Fig. 2).

The embryo envelope in orchid is similar in some ways to the embryo envelope in seeds of Cucurbitaceae. The apoplastic semipermeable envelope in *Cucumis melo* is caused by deposition of a thick callose-containing layer outside the endosperm cell walls, apparently analogous to the carapace in orchid seeds (Yim and Bradford, 1998). In cucurbit seeds, this envelope has likely evolved to protect embryos from the harsh environment in decaying fruit and soil. The embryo envelope in *C. melo* produces tension against embryo expansion that is broken when the endosperm envelope is enzymatically degraded during radicle emergence, establishing a gradient for new water uptake driving expansive growth of the radicle (Welbaum and Bradford, 1990). Cutinization and lignification has been suggested to strengthen the inner integument, and the carapace could inhibit embryo expansive growth by mechanical restriction as well (Miyoshi and Sato, 1997). The presence of the hydrophobic carapace cuticle in orchid may help seeds survive in harsh environments (Lee et al., 2005) and protect the embryo from desiccation (Yeung et al., 1996). An embryo envelope with properties similar to orchid has also been characterized in *Lactuca sativa* (Hill and Taylor, 1989).

Seed contamination was not a significant problem in 90–120 DAP developing seeds in this study because pods were harvested intact and seeds were not exposed to epiphytic bacteria and other potential contaminants. With the exception of 5% treatments for 5 min, all CH seed treatments adversely affected protocorm development of 90 DAP seeds (Fig. 5). CH may be most effective on seeds harvested from senescing pods with more surface contamination or resilient testae. Fully mature seed lots from Taiwan were less sensitive to 15% CH than the developing seeds that were easily damaged by short-term exposure to higher concentrations (cf. Table 3, Figs. 5 and 6).

Our results conflict with findings that CH stimulated orchid seed germination. Vujanovic et al. (2000) observed an increase in the germinability of *Cypripedium* spp. after treatment. In *J. effusus*, Ervin and Wetzel (2002) found that seeds germinated faster if treated with CH. It is likely that CH efficacy depends on the species or seed maturity. In immature seeds that are highly permeable, embryos may be easily damaged by relatively low concentrations and short exposure times. In mature seeds or species with a well-developed testa and carapace, CH may improve germination by scarification. According to Hicks (2004), *Phalaenopsis* viability drops sharply with extended exposure times or high concentrations of hypochlorite and the observed negative effect is a function of pH instead of actual chlorine concentration. Surface sanitization of mature green pods with 15% CH was sufficient to eliminate any significant contamination in culture and had no effect on seed vigor in this study. Directly treating seeds with <5% CH risks damaging sensitive developing orchid embryos.

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